

Original Research Article

TOXICITY INDUCED HAEMATOLOGICAL ALTERATIONS AFTER ACUTE AND CHRONIC ADMINISTRATION OF TARTRAZINE (E102) IN ALBINO RATS

ABSTRACT

Aim: To evaluate the Haematological alterations induced by tartrazine after acute and chronic administration in albino rats.

Study design: The design involved acute and chronic study. The acute study investigated intraperitoneal and oral route of administration while the chronic study used oral route only. The rats used weighed 0.15kg approximately. In the acute study, 48 rats (24 female and 24 male) were used for intraperitoneal treatment and were randomly selected and placed into six groups treated with 0.0g/kg, 1.67g/kg, 3.33g/kg, 5.0g/kg, 6.67g/kg and 8.33g/kg of tartrazine. In orally treated rats, 48 rats (24 female and 24 male) were also used and were treated with 0.0g/kg, 2.5g/kg, 5.0g/kg, 10.0g/kg, 15.0g/kg and 20.0g/kg of tartrazine. In the chronic study, the experiment was divided into phase 1, 2 and 3 which lasted for 30, 60 and 90 days respectively. In each phase, 80 rats were used and were divided into treatment and control groups. The treated groups were given 7.5mg/kg of tartrazine orally on daily basis over the stipulated periods while the control groups were not treated with tartrazine.

Place and Duration of Study: The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria within a period of 12 months (December, 2017 – December, 2018).

Methodology: At the end of the acute and chronic study, 5mls of whole blood specimens was collected by means of cardiac puncture into K₃EDTA bottles. The collected specimens were analysed immediately using Mindray 5300 haematology autoanalyzer. Statistical analysis was performed using GraphPad Prism version 5.03 (San Diego, California, USA).

Results: In acute toxicity study, the administration of high doses far above the ADI of tartrazine induced decreased RBCs, HB, HCT, WBCs, Eosinophil and Neutrophil counts as well as an increased PLTs, Lymphocyte and Monocyte counts. However, in the chronic treatment, WBCs were increased after 60 and 90 days of chronic treatment at ADI doses while Eosinophil and Basophil counts showed significant decrease after 30 and 60 days of treatment. Also, increase in Lymphocyte was observed after 30, 60 and 90 days. In addition, Neutrophil and Monocyte counts showed significant lower levels after 30, 60 and 90 days of chronic treatment with tartrazine. HCT, HB, PLTs showed no significant difference after 30, 60 and 90 days of chronic treatment at ADI doses.

Conclusion: The results obtained indicate that high doses of tartrazine above the recommended ADI induced severe haematological alterations. However, the chronic study did not affect HCT, HB and PLTs but mild derangements/alterations were in WBCs, lymphocyte, Neutrophil, Eosinophil and Basophil counts after 60 and 90 days of treatment.

Keywords: Tartrazine, haematological parameters, acute study, chronic study, albino rats

1. INTRODUCTION

Tartrazine is a synthetic food dye commonly used in many food and food products to enhance the appearance of such food products [1], [2]. Tartrazine has been reported to cause or induce several clinical derangements when consumed in excess or even at

recommended dose [2], [3], [4]. The toxicity of synthetic dyes such as tartrazine has been linked to the reductive biotransformation of the azo bond during their metabolism in the intestine and liver producing reactive amines, aryl amines and free radicals [5]. In this study, the influence of tartrazine on haematological parameters of rats was analyzed.

Haematological studies entail the study of blood cells and their abnormalities as it affects their production from the bone marrow [6]. Blood consist of erythrocytes, leukocytes and thrombocytes that are suspended in fluid called plasma [7]. In clinical practice, Full Blood Count (FBC) is usually assayed to assess one's health [6], [8]. Component of the FBC considered in this study include, haemoglobin (Hb) concentration, haematocrit (HCT), Red Blood Cells (RBC), Platelet (PLT), White Blood Cells (WBCs) count and percentage differential leucocytes count [6], [8], [9].

Haemoglobin (Hb) is an iron-containing macromolecule contained in RBCs and is mainly involved in transporting oxygen to tissues and carbon dioxide out of tissue via the lungs [6], [7]. Hb and RBCs levels vary with age, sex and geographical location and they important in the diagnosis of anaemia or polycythemia [6], [9]. Aboel-Zahab et al. [10], Sharma et al.[11], reported in their separate studies that tartrazine induced a significant decrease in RBCs and Hb content in rats. However, Mehedi et al. [12], reported increased red blood cell and Hb when tartrazine was fed to rats. As defined by Baker et al. [13], haematocrit (HCT) is the relative mass of red blood cells present in a sample of whole blood. It gives a diagnostic insight of the oxygen carrying capacity of the blood and the erythropoietic state (whether anaemic or polycythemic) of the reticulo-endothelial system and it is also sex, age and geographical location dependent [6], [8]. In a study, Daffallah and colleagues [3], reported tartrazine at a dose of 0.1g/kg bodyweight induced a significant decrease in RBCs count, Hb and HCT in rats when administered in rats at interval of 2 days for 12 weeks. However, Mehedi and colleagues [12], stated in their study that HCT values were increased when tartrazine was fed orally to rats at a dose of 2.5% for 13 weeks.

White blood cells (WBCs) also known as Leukocytes are nucleated blood cells divided into granulocytes and agranulocytes [7]. Neutrophils, eosinophils and basophils are classified as granulocytes while monocytes and lymphocytes are agranulocytes [7]. The main function of WBCs is to act as one of the body defense [6], [7], [9]. When the total leukocyte count falls significantly below normal, it is termed leukopenia and leukocytosis when the count increases significantly above normal [6]. Factors such as xenobiotics, infections, trauma, among others have reported to induce leukopenia or leucocytosis [6], [14]. In separate studies carried out by Daffallah et al. [3] and Sharma et al. [11], it was stated that tartrazine dye at high doses when orally fed to rats caused significant decrease in leukocyte count. However, Hashem and colleagues [15], reported that administration of tartrazine for 35 days at dosage of 315mg/kg bodyweight did not change the WBCs count. More so, Himri and colleagues [16], also reported that administration of tartrazine for 90 days at dosage of 5.0, 7.5 and 10mg/kg bodyweight did not affect the WBCs count. WBCs consist of different cells types that can be differentiated using the differential white cell count technique.

Differential white cell count is the means of identifying the different white cells type and establishing their counts per 100 white blood cells [8]. Differential count is important since some pathological conditions such as urinary tract infection, certain drugs, allergies, parasitic infections, among others that might not significantly affect the total leukocyte count might grossly affect one or more cell type affecting their number, size and shape [17], [18].

Neutrophils are the most abundant white blood cells in peripheral blood smear [18]. They have distinct cytoplasm and nucleus. Their cytoplasm contains tiny granules and the nucleus in made of 3 – 6 lobes connected by a thin strand of chromatin [17]. Several etiological

agents such as tuberculosis, toxic conditions, malignancies, certain drugs, etc have been reported to cause neutropenia or neutrophilia as well as reduced or increased number of lobes [18]. In a study carried out by Sharma et al. [11], it was reported that tartrazine at high doses induced a significant decrease in neutrophils count when fed to rats for 35 days. However, Himri and colleagues [16], reported a significant increase in neutrophil at a dose of 10mg/kg. it was further stated that at a dose of 7.5mg/kg, neutrophilic counts were not affected. Another WBCs type is the lymphocytes. Lymphocytes play vital role in active and passive immunity through the T-lymphocytes and B-lymphocytes. The B-lymphocyte differentiates to form plasma cells concerned with antibody production while T-lymphocytes are mainly activated in the thymus and are involved in cell-mediated immunity [18]. Agents such as tuberculosis, hepatitis B, toxins, xenobiotics, etc have been recorded to induce lymphocytosis or lymphopenia [18], [19]. In a study carried out by El-Golli et al., [20], it was recorded that 300mg/kg of tartrazine dye fed to rats for 30 days induced a significant decrease in lymphocytic counts in male adult rats. Imafidon et al. [21], also reported that tartrazine at high dose of 80mg/kg did not induce any change in neutrophilic or lymphocytic cell count in rats.

Monocytes are the largest of leukocytes in peripheral blood [22]. Monocytes are principally phagocytic leukocytes that can ingest a whole organism or foreign materials e.g. Kuffer cells of the kidney [22]. Eosiniphils are granulocytic white cells which play vital role in fighting infection by removing antigen-antibody complexes via phagocytic means [7], [23]. Significant increase in eosinophilic count is termed eosinophilia and this may be seen in allergic reactions, parasitaemia, hypersensitivity skin disorders and leukaemia [17], [18]. Basophils are also granulocytic white cells with a distinct nucleus containing two or more lobes slightly occluded due to large granules contained in the cytoplasm. These granules contain histamine and heparin that are involved in inflammatory and immunologic processes [17], [24]. Increased basophilic count is termed basophilia and basophilia may be rare or occasional [24]. In a study carried out by Sharma et al.[11], it was recorded that tartrazine at a dose of 0.2 and 0.4g/kg administered in rats for 35 days did not induced changes in monocytic, eosinophilic and basophilic counts.

The platelets are derived from parent cells called megakaryotes [25], [26]. They are mainly involved in maintenance of haemostasis and integrity of blood vessels [25]. Platelets are also used to assess toxicity of chemical and pathological changes in an individual [8], [25], [27]. In a study carried out by El-Golli et al. [20], it was recorded that tartrazine at a dose of 300mg/kg fed to rats for 30days induced significant increase in platelet count.

Tartrazine commonly used as food dye has been reported in different literatures to cause several clinical derangements such as attention deficit, hepatic and nephrotic derangements, among others when consumed in excess or even at recommended dose [3], [28]. The effect of synthetic dyes such as tartrazine on haematological parameters still remains controversial in most scientific studies. Therefore, this study was designed to look at acute and chronic toxicity of tartrazine dye on haematological parameters.

2. MATERIALS AND METHODS

2.1 Materials

Materials used in this study includes K₃EDTA bottles, polypropylene gavage tubes (Intech Laboratory Incorporated, Plymouth Meeting, USA), Haier thermocool refrigerator (China), Ohaus Scout-Pro Electronic weigh balance (Ohaus Corporation, New Jersey, USA), Albino rats, Mindray BS5300 haematology, autoanalyser, Tartrazine dyes (CI. 19140, CAS No 1934-21-0, MW 534,37, E102, FD& C NO 5) with serial no. of F119371 purchased in a

granular form from Fiorio Colori Spa, Gessete, Italy, with purity of 86.7%. Other materials used include automatic pipettes, hypodermic syringe and chloroform.

2.2 Experimental Animals

Male and female albino rats weighing approximately 0.15kg were used for the experiment. All the rats used for the experiment were obtained by breeding. However, the parent rats used for the breeding were purchased from the University of Port Harcourt, River State, Nigeria. The rats were fed with chicken grower's mash and water *ad libitum* in well-ventilated cages.

2.3 Preparation of Tartrazine Dye

In the acute study, for intraperitoneal treatment, 250g of the tartrazine was dissolved in a sterile container containing 1 litre of distilled water. This implies that 1.0ml of this solution contains 0.25 grams. In terms of oral treatment (acute study), 375g of the tartrazine dyes was also dissolved in sterile containers containing 1 litre of distilled water. This implies that 1.0ml of this solution contains 0.375g of tartrazine. Finally, in the chronic study, 1.13 grams of tartrazine was weighed and dissolved in a sterile container containing 1.0 litre of distilled water. This implies that, 1.0ml of the tartrazine solution contains 0.00113g, which is equivalent to 7.5mg/kg when administered into a 0.15kg rat. The contents of the containers were properly mixed to ensure complete mixture before administration.

2.4 Experimental Design and Administration of Dye

The method of treatment in the acute studies involved both intraperitoneal and oral techniques while in the chronic study, treatment was strictly orally. In the intraperitoneal method, the dyes were injected into the intraperitoneal space of the rats using 2 ml and 5 ml hypodermic syringes while in the oral method, the food dyes were administered using gavage tube to ensure complete delivery of the dye.

2.4.1 Acute treatment

Dose range in both oral and intraperitoneal treatment were determined after the obtaining the value of LD₅₀ using the arithmetic method of Karber as described by Dede et al. [29]. The LD₅₀ was calculated to be 5.83g/kg and 11.25g/kg for intraperitoneal and orally treated rats respectively. In the intraperitoneal treatment, 48 rats (24 male & 24 female rats) were used. The male and female rats were randomly selected and placed into six different groups separately designated as A_{TIP} (control), B_{TIP}, C_{TIP}, D_{TIP}, E_{TIP} and F_{TIP} and were treated with 0.0g/kg, 1.67g/kg, 3.33g/kg, 5.0g/kg, 6.67g/kg and 8.33g/kg of tartrazine respectively. In terms of orally treated rats, 48 rats (24 males; 24 females) were also used. The male and female rats were randomly selected and placed into six different groups separately. The groups were designated as A_{TO} (control), B_{TO}, C_{TO}, D_{TO}, E_{TO} and F_{TO} and were orally treated with 0.0g/kg, 2.5g/kg, 5.0g/kg, 10.0g/kg, 15.0g/kg and 20.0g/kg of tartrazine respectively.

2.4.2 Chronic Treatment

The chronic treatment was performed over a period (phase) of 30, 60 and 90 days. Eighty (80) experimental rats weighing approximately 0.15kg were used in each phase of the study (with a total of 119 females and 116 male rats of which 5 died in course of the experiment). In each phase, the rats were divided into two groups designated T_T (tartrazine treated group), and C (control, untreated group). Rats in each of these groups were further distributed randomly into 10 cages with four rats per cage, designated T_{T1}, T_{T2}...T_{T10}. In the

treatment pattern, the acceptable daily intake (ADI) of 7.5mg/kg of tartrazine was administered orally. The control groups were not administered with tartrazine.

2.5 Study Area

The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt. However, whole blood samples collected with K₃EDTA bottles were immediately transported (45-minute drive) to the Haematology Unit, University of Port Harcourt Teaching Hospital where all the haematological parameters were analysed using Mindray BS 5300 haematology autoanalyser.

2.6 Specimen Collection, Preparation and Analysis

At the end of the study, the animals were anaesthetized with chloroform and 5mls of whole blood samples was collected by means of cardiac puncture into K₃EDTA bottles. The collected whole blood samples were properly mixed to ensure adequate anticoagulation before the assay of haematological parameters. The whole blood specimens collected were assayed immediately using Mindray BS5300 haematology auto-analyzer.

2.7 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.03 (San Diego, California, USA). Results were presented as Mean \pm Standard deviation (SD). Inferential statistics using Students' statistical t-test was employed to compare values of the treated rats and control rats. In addition, the One-Way ANOVA (Post Hoc: Tukey's multiple comparative test) was also used to evaluate the influence of treatment dosage in the acute study. Statistical significance was set at $P=0.05$.

3. RESULTS

3.1 Results of Acute Study on Haematological Parameters in Rats Treated with Tartrazine Intraperitoneally

Table 1a shows significantly lower values in HCT, HB and RBC in treated rats in all the dosages compared with control rats. However, significantly higher values were seen in PLT in rats compared with control at dose 8.33g/kg at $P=0.05$. Table 1b shows that WBC, Neutrophils, Eosinophils and Basophils values were significantly lower in treated rats compared with control at 3.33g/kg to 8.33g/kg while Lymphocytes showed significantly higher values in treated rats compared with control at 3.33g/kg to 8.33g/kg. Mono indicated significantly higher values in treated rats compared with control at dose 5.0g/kg to 8.33g/kg at $P=0.05$.

Table 1a. Haematological Parameters in Rats Treated with Tartrazine Intraperitoneally

Parameters	HCT (%)	HB (g/dl)	RBC $\times 10^{12}/L$	PLT $\times 10^9/L$
0.0g/kg (A _{TIP} ;Control)	49.43 \pm 5.89 ^a	16.15 \pm 2.03 ^a	10.37 \pm 0.78 ^a	309.5 \pm 218.9 ^a
1.67g/kg (B _{TIP})	29.70 \pm 11.28 ^{bc}	9.83 \pm 3.83 ^{bc}	5.43 \pm 2.29 ^{bc}	521.0 \pm 86.03 ^{ac}
3.33g/kg (C _{TIP})	31.35 \pm 2.37 ^{bcd}	10.25 \pm 0.77 ^{bcd}	5.81 \pm 0.59 ^{bcd}	602.5 \pm 27.77 ^{acd}

5.0g/kg (D _{TIP})	38.18±4.54 ^{acde}	12.60±1.54 ^{acde}	6.94±0.94 ^{acde}	606.5±115.5 ^{acde}
6.67g/kg (E _{TIP})	38.53±3.11 ^{acdef}	12.45±0.93 ^{acdef}	7.42±1.13 ^{acdef}	644.5±160.7 ^{acdef}
8.33g/kg (F _{TIP})	34.45±2.82 ^{bcdef}	10.83±0.33 ^{bcdef}	7.64±1.49 ^{acdef}	918.8±46.38 ^{bcdef}
P value	0.0023	0.0025	0.0009	0.0220
F value	5.830	5.703	7.007	3.497
Remark	S	S	S	S

Values in the same column with different superscript letter (a, b) differ significantly when comparing the control with other groups. Values in the same column with same superscript letter (c, d, e and f) do not differ significantly when comparing the group B_{TIP} C_{TIP} D_{TIP} E_{TIP} and F_{TIP} with one another. No of Rats/group = 4 Rats.

Table 1b. Haematological Parameters of Rats Treated with Tartrazine Intraperitoneally

Parameters	WBC×10 ⁹ /L	N (%)	L (%)	M (%)	E (%)	B (%)
0.0g/kg (A _{TIP} ; cOntrol)	19.28±7.47 ^a	20.98±6.31 ^a	62.90±3.84 ^a	12.97±1.54 ^a	2.70±1.61 ^a	0.48±0.26 ^a
1.67g/kg (B _{TIP})	12.40±5.02 ^{ac}	15.90±4.54 ^{ac}	67.45±4.12 ^{ac}	13.65±1.61 ^{ac}	2.43±0.95 ^{ac}	0.33±0.22 ^{ac}
3.33g/kg (C _{TIP})	7.38±6.22 ^{bcd}	11.33±1.42 ^{bcd}	73.90±3.13 ^{bcd}	17.55±4.67 ^{acd}	0.30±0.20 ^{bcd}	0.03±0.02 ^{bcd}
5.0g/kg (D _{TIP})	8.80±3.54 ^{acde}	12.88±1.51 ^{bcde}	69.02±2.74 ^{acde}	16.93±1.97 ^{acde}	0.68±0.30 ^{bcde}	0.13±0.05 ^{bcde}
6.67g/kg (E _{TIP})	9.75±1.13 ^{acdef}	11.13±1.13 ^{acdef}	69.63±3.46 ^{acdef}	18.63±4.14 ^{acdef}	0.58±0.21 ^{bcdef}	0.05±0.03 ^{bcdef}
8.33g/kg (F _{TIP})	8.10±1.79 ^{bcdef}	8.90±3.21 ^{acdef}	71.58±4.92 ^{bcdef}	18.48±1.99 ^{bcdef}	0.43±0.21 ^{bcdef}	0.08±0.05 ^{bcdef}
Pvalue	0.0230	0.0021	0.0133	0.0476	0.0006	0.0024
Fvalue	3.457	5.903	3.969	2.815	7.551	5.761
Remark	S	S	S	S	S	S

Values in the same column with different superscript letter (a, b) differ significantly when comparing the control with other groups. Values in the same column with same superscript letter (c, d, e and f) do not differ significantly when comparing the group B_{TIP} C_{TIP} D_{TIP} E_{TIP} and F_{TIP} with one another. No of Rats/group = 4 Rats.

3.2 Results of Acute Study on Haematological Parameters in Rats Orally Treated with Tartrazine

Table 2a shows significantly lower values in HCT, HB and RBC from dose 2.5g/kg in other groups compared with control while significantly higher values were seen in PLT from dose 2.5g/kg compared with control at $P=0.05$. Table 2b shows that WBC and Neutrophils were significantly lower in treated rats compared with control rats from dose 5.0g/kg while

Eosinophils and Basophils also showed significantly lower values in treated rats compared with control from dose 10.0g/kg and 15.0g/kg respectively. Finally, significantly higher values were seen in Lymphocytes in treated rats compared with control at 20.0g/kg at $P=0.05$.

Table 2a: Haematological Parameters of Rats Administered with Tartrazine Orally

Parameters	HCT (%)	HB (g/dl)	RBC $\times 10^{12}/L$	PLT $\times 10^9/L$
0.0g/kg (A _{TO} ; Control)	49.43 \pm 5.89 ^a	16.15 \pm 2.03 ^a	10.37 \pm 0.78 ^a	309.5 \pm 218.9 ^a
2.5g/kg (B _{TO})	33.40 \pm 5.49 ^{bc}	10.73 \pm 1.79 ^{bc}	6.27 \pm 0.95 ^{ac}	968.3 \pm 88.83 ^{bc}
5.0g/kg (C _{TO})	35.48 \pm 1.99 ^{bcd}	11.63 \pm 0.48 ^{bcd}	6.60 \pm 0.37 ^{acd}	707.0 \pm 165.8 ^{acd}
10.0g/kg (D _{TO})	35.83 \pm 6.0 ^{bcde}	12.10 \pm 2.02 ^{bcde}	4.67 \pm 3.75 ^{bcde}	944.0 \pm 325.0 ^{bcde}
15.0g/kg (E _{TO})	35.48 \pm 5.78 ^{bcdef}	11.40 \pm 1.90 ^{bcdef}	4.90 \pm 2.70 ^{bcdef}	1049.0 \pm 159.5 ^{bcdef}
20.0g/kg (F _{TO})	37.70 \pm 4.32 ^{bcdef}	11.58 \pm 1.71 ^{bcdef}	6.68 \pm 0.59 ^{bcdef}	674.0 \pm 100.5 ^{acdef}
Pvalue	0.0041	0.0045	0.0094	0.0004
Fvalue	5.176	5.071	4.311	7.944
Remark	S	S	S	S

Values in each column with different superscript letter (a, b) differ significantly when comparing the control (A_{TO}) with other groups. Values in the same column with same superscript letter (c, d, e and f) do not differ significantly when comparing the groups B_{TO}, C_{TO}, D_{TO} and E_{TO} with one another. No of Rats/group = 4 Rats

Table 2b. Haematological Parameters in Rats Treated Orally with Tartrazine

Parameters	WBC $\times 10^9/L$	N (%)	L (%)	M (%)	E (%)	B (%)
0.0g/kg (A _{TO} ; Control)	19.28 \pm 7.47 ^a	20.98 \pm 6.31 ^a	62.90 \pm 3.84 ^a	12.97 \pm 1.54 ^a	2.70 \pm 1.61 ^a	0.48 \pm 0.26 ^a
2.5g/kg (B _{TO})	17.60 \pm 5.93 ^{ac}	14.18 \pm 5.99 ^{ac}	69.0 \pm 6.89 ^{ac}	13.65 \pm 0.98 ^{ac}	2.78 \pm 1.54 ^{ac}	0.40 \pm 0.35 ^{ac}
5.0g/kg (C _{TO})	8.76 \pm 2.81 ^{bcd}	11.30 \pm 1.45 ^{bcde}	72.80 \pm 3.67 ^{bcd}	13.18 \pm 1.67 ^{acd}	1.45 \pm 0.40 ^{acd}	0.23 \pm 0.05 ^{acd}
10.0g/kg (D _{TO})	8.96 \pm 1.42 ^{bcde}	13.65 \pm 0.98 ^{ac}	72.33 \pm 5.19 ^{bcde}	15.93 \pm 3.23 ^{acde}	0.48 \pm 0.29 ^{bcde}	0.23 \pm 0.10 ^{acde}
15.0g/kg (E _{TO})	9.88 \pm 1.57 ^{bcdef}	11.63 \pm 2.54 ^{bcdef}	71.43 \pm 3.24 ^{bcdef}	16.08 \pm 2.40 ^{acdef}	0.68 \pm 0.17 ^{bcdef}	0.13 \pm 0.05 ^{bcdef}
20.0g/kg (F _{TO})	7.30 \pm 0.58 ^{bcdef}	10.43 \pm 0.71 ^{bcdef}	72.05 \pm 2.91 ^{bcdef}	19.23 \pm 4.79 ^{bcdef}	0.45 \pm 0.24 ^{bcdef}	0.10 \pm 0.14 ^{bcdef}
P value	0.0019	0.0121	0.0487	0.0350	0.0036	0.0771

F value	6.058	4.062	2.796	3.082	5.324	2.409
Remark	S	S	S	S	S	S

Values in each column with different superscript letter (a, b) differ significantly when comparing the control (A_{TO}) with other groups. Values in the same column with same superscript letter (c, d, e and f) do not differ significantly when comparing the groups B_{TO}, C_{TO} D_{TO} and E_{TO} with one another. No of Rats/group = 4 Rats

3.3 Results of Haematological Parameters in Rats Chronically Treated with Tartrazine Over a period of 30 Days

When both male and female tartrazine treated rats were compared with their respective control rats, Neutrophils and Eosinophils showed a significantly lower value in tartrazine treated rats compared with control rats. Lymphocytes also indicated a significantly higher value in tartrazine treated rats compared with control rats at $P=0.05$ (table 3a and table 3b).

Table 3a. Haematological Parameters in Male Rats Chronically Treated with Tartrazine Over a Period of 30 Days

Parameters	Control Rats (Males) n=18	Treated Rats (Males) n=17	P value	T value	Remark
HCT (%)	37.67±8.16	35.49±9.46	0.4703	0.7304	NS
HB (g/dl)	11.43±2.12	10.73±2.74	0.3928	0.8659	NS
RBC(x10 ¹² /L)	6.04±5.82	5.82±1.41	0.6322	0.4831	NS
PLT (x10 ⁹ /L)	428.3±260.7	538.1±290.9	0.2475	1.177	NS
WBC(x10 ⁹ /L)	7.91±9.86	8.10±4.73	0.8978	0.1295	NS
N (%)	34.61±9.27	26.88±11.55	0.0359	2.188	S
L (%)	52.27±13.94	64.81±10.23	0.0049	3.019	S
M (%)	7.81±3.31	5.66±4.24	0.1035	1.675	NS
E (%)	4.38±3.44	1.55±0.90	0.0025	3.277	S
B (%)	0.56±0.36	0.51±0.29	0.6528	0.4510	NS

n= no of Rats, NS= Not Significant, S= Significant

Table 3b. Haematological Parameters in Female Chronically Treated with Tartrazine Over a Period of 30 Days

Parameters	Control Rats (Females) n=22	Treated Rats (Females) n=22	P value	T value	Remark
HCT (%)	39.02±6.83	37.34±8.43	0.4711	0.7273	NS
HB (g/dl)	11.74±1.62	11.22±2.43	0.4055	0.8403	NS
RBC(x10 ¹² /L)	6.21±1.03	6.01±1.25	0.5573	0.5916	NS
PLT (x10 ⁹ /L)	446.1±259.6	479.7±298.7	0.6929	0.3976	NS
WBC(x10 ⁹ /L)	7.95±4.29	8.18±4.73	0.8683	0.1669	NS
N (%)	32.92±10.72	25.48±12.83	0.0429	2.088	S
L (%)	55.19±15.04	64.86±10.30	0.0169	2.489	S
M (%)	7.66±3.25	7.0±4.63	0.5547	0.5821	NS
E (%)	4.16±3.90	1.58±1.03	0.0045	3.006	S
B (%)	0.44±0.28	0.48±0.35	0.6337	0.4800	NS

n= no of Rats, NS= Not Significant, S= Significant

3.4 Results of haematological Parameters in Rats Chronically Treated with

Tartrazine Over a Period of 60 Days

When male control and male treated rats were considered, Neutrophils and Monocytes showed significantly lower values in tartrazine male treated rats compared with male control rats. Lymphocytes indicated a significant higher value in tartrazine treated male rats compared with a male control rat at $P=0.05$ (table 4a). When tartrazine treated female rats and control female rats were considered, WBC (White Blood Cells) indicated a significantly higher value in tartrazine treated female rats compared with control rats. Monocytes indicated a significantly lower value in tartrazine treated female rats compared with control female rats at $P=0.05$ (table 4b).

Table 4a. Haematological Parameters of Male Rats Chronically Treated with Tartrazine Over a Period of 60 Days

Parameters	Control Rats (Males) n=20	Treated Rats (Males) n=25	P value	T value	Remark
HCT (%)	41.17±2.98	39.69±2.42	0.0732	1.837	NS
HB (g/dl)	12.73±0.93	12.25±0.82	0.0733	1.836	NS
RBC(x10 ¹² /L)	6.78±0.49	6.40±0.94	0.1073	1.645	NS
PLT (x10 ⁹ /L)	597.7±163.2	565.2±266.2	0.6355	0.4774	NS
WBC(x10 ⁹ /L)	9.52±5.69	8.40±3.72	0.4334	0.7909	NS
N (%)	33.92±7.92	26.0±10.30	0.0084	2.764	S
L (%)	54.45±8.68	66.38±9.31	<0.0001	4.406	S
M (%)	7.06±2.58	4.41±2.62	0.0015	3.388	S
E (%)	3.18±2.30	2.84±1.35	0.5387	0.6193	NS
B (%)	0.41±0.27	0.38±0.22	0.6396	0.4716	NS

n= no of Rats, NS= Not Significant, S= Significant

Table 4b. Haematological Parameters of Female Rats Chronically Treated with Tartrazine for 60 Days

Parameters	Control Rats (Females) n=20	Treated Rats (Females) n=15	P value	T value	Remark
HCT (%)	32.38±12.30	32.30±9.64	0.9845	0.0195	NS
HB (g/dl)	10.74±3.56	10.60±2.76	0.9003	0.1263	NS
RBC(x10 ¹² /L)	5.03±1.80	6.11±1.24	0.0544	1.995	NS
PLT (x10 ⁹ /L)	550.6±257.2	543.6±169.6	0.9283	0.0907	NS
WBC(x10 ⁹ /L)	3.17±1.39	8.28±4.59	<0.0001	4.718	S
N (%)	28.6±10.79	28.38±8.26	0.9491	0.0643	NS
L (%)	62.22±11.43	64.23±8.75	0.5731	0.5692	NS
M (%)	7.26±3.15	4.95±3.15	0.0391	2.148	S
E (%)	1.97±1.58	2.76±2.20	0.2233	1.241	NS
B (%)	0.39±0.22	0.32±0.28	0.9061	0.1189	NS

n= no of Rats, NS= Not Significant, S= Significant

3.5 Results of Haematological Parameters of Rats Chronically Treated with Tartrazine Over a Period of 90 Days

When tartrazine treated male rats were considered, WBCs and Lymphocytes indicated a significantly higher value in treated male rats compared with control male rats. Neutrophil

and Basophils showed a significantly lower value in tartrazine treated male rats compared with control male rats at $P=.05$ (table 5a). When tartrazine treated female rats were considered, Neutrophils, Monocytes and Basophils showed a significantly lower value in tartrazine treated female rats compared with control female rats. Lymphocytes indicated a significantly value in treated female rats compared with control female rats at $P=.05$ (table 5b).

Table 5a. Haematological Parameters of Male Rats Chronically Treated with tartrazine Over a Period 90 Days

Parameters	Control Rats (Males) n=19	Treated Rats (Males) n=17	P value	T value	Remark
HCT (%)	40.19±5.01	42.04±9.23	0.4540	0.7574	NS
HB (g/dl)	12.33±1.92	12.58±2.63	0.7502	0.3210	NS
RBC(x10 ¹² /L)	6.76±0.77	7.06±1.98	0.5451	0.6112	NS
PLT (x10 ⁹ /L)	468.1±241.7	578.6±283.6	0.2213	1.246	NS
WBC(x10 ⁹ /L)	7.54±3.83	10.28±4.06	0.0448	2.083	S
N (%)	36.51±8.63	27.28±6.70	0.0011	3.553	S
L (%)	51.98±11.88	62.89±7.61	0.0027	3.236	S
M (%)	7.33±3.05	6.25±2.58	0.2645	1.135	NS
E (%)	2.90±2.32	3.19±1.44	0.6611	0.4423	NS
B (%)	0.55±0.42	0.24±0.12	0.0058	2.945	S

n= no of Rats, NS= Not Significant, S= Significant

Table 5b. Haematological Parameters of Female Rats Chronically Treated with Tartrazine Over a Period 90 Days

Parameters	Control Rats (Female) n=18	Treated Rats (Female) n=22	P value	T value	Remark
HCT (%)	39.19±4.68	39.33±6.89	0.9407	0.0749	NS
HB (g/dl)	12.25±1.43	12.01±2.44	0.7135	0.3699	NS
RBC(x10 ¹² /L)	6.06±1.19	6.42±1.69	0.4605	0.7456	NS
PLT (x10 ⁹ /L)	397.6±256.7	520.8±160.9	0.1088	1.642	NS
WBC(x10 ⁹ /L)	6.48±3.18	8.35±3.98	0.1154	1.6110	NS
N (%)	34.24±9.55	25.89±6.76	0.0025	3.236	S
L (%)	52.74±14.37	65.36±7.39	0.0009	3.588	S
M (%)	8.89±3.63	5.23±1.52	0.0001	4.301	S
E (%)	3.71±3.01	3.49±1.85	0.7785	0.2833	NS
B (%)	0.58±0.30	0.37±0.25	0.0177	2.479	S

n= no of Rats, NS= Not Significant, S= Significant

4. DISCUSSION

Tartrazine has been reported to induce several clinical derangements when consumed in excess or even at recommended dose [3], [4]. Therefore, the study looked at acute and chronic toxicity of tartrazine dye on haematological parameters.

In the acute study, significantly lower values were seen in HCT, HB, RBC, WBCs, Neutrophils, Eosinophils and Basophils while significantly higher values were observed in PLT, Monocytes and Lymphocytes in rats treated intraperitoneally and orally at varying dosages when compared to control rats. The results of HCT, HB and RBCs support the reports of Daffallah et al. [3], Aboel-Zahah et al. [10] and Sharma et al.[11]. Daffallah et al.,

[3], reported that tartrazine at a dose of 0.1g/kg bodyweight induced a significant reduction in HB and RBCs in rats treated for 12 days when administered at intervals of 2 days. Aboel-Zahah et al. [10], reported tartrazine induced a significant decrease in RBCs and HB in rats supplemented with carmoisine and brilliant blue. More so, Sharma et al. [11], reported that tartrazine at a dose of 0.2g/kg and 0.4g/kg bodyweight induced significant reduction in HB and RBCs when rats were treated for 35days. On the contrary, Mehedi and colleagues [12], reported that high doses (2.5%) of tartrazine administered daily for 13 weeks caused significant increase in HCT, HB and RBCs of albino rats which was attributed mainly to induced dehydration. In addition, Imafidon et al.[21], reported significant increase in HCT only when tartrazine was administered in rats at a dose of 80mg/kg on weekly basis for 4 weeks. It was also reported that lower doses (10, 20 and 40mg/kg) of tartrazine did not induce any change in RBCs, HCT and HB in treated rats compared to control. The significantly lower values seen in HCT, HB and RBCs in both intraperitoneal and orally treated rats suggest possible development of anaemia which could have been caused by RBCs destruction induced by membrane distortion as well as the preventing of iron supply/absorption for haemoglobin synthesis as a result of negative pharmacological interaction of azo dyes and iron in the gastrointestinal mucosal lining of the rats. The induced distortion of the red cells membrane probably further enhanced the destruction and removal of these cells by the reticulo-endothelial system (RES) thereby resulting in reduced RBCs, HCT and HB.

The significantly higher PLT values observed in the acute study concurs with report of Himri et al., [16], El-Golli et al.[20]. Himri et al. [16], reported significant increase in platelet count when rats were treated with tartrazine at a dose slightly above ADI (10mg/kg) for 90 days. In addition, El-Golli et al. [20], also reported significant increase in peripheral platelet count when male albino rats were treated with tartrazine at a dose of 300mg/kg bodyweight for 30 days. The significantly higher values of PLT count observed in our study could be due to physiological inflammatory body response to tissue damages or micro-tears induced by tartrazine.

More so, significant reduction in WBCs was also seen in the acute study. The significantly reduced values in WBCs observed in our study collaborates with the findings of [3], [11], [12] but contrast the findings of [15], [16]. Daffallah et al. [3], reported that tartrazine at a dose of 0.1g/kg bodyweight induced a significant reduction in WBCs in rats treated at interval of 2days for 12 days. In addition, Sharma et al. [11], recorded significant reduction in WBCs when rats were treated for 35days with tartrazine at a dose of 0.2g/kg and 0.4g/kg bodyweight. More so, Mehedi et al.[12], also reported that high doses (2.5%) of tartrazine above ADI caused significant decrease in WBCs of treated albino rats compared to control. However, Hashem et al. [15], observed no significant difference in WBCs when rats were treated with tartrazine at a dose as high as 315mg/kg for 4 weeks. Also, Himri et al., [16], reported no significant difference in WBCs when rats were treated with tartrazine at a dose slightly above ADI (10mg/kg) for 90 days. In addition, significantly lower values were also observed in Neutrophilic count. The significant neutropenia seen in treated rats also agrees with the findings of [11] but contradicts the findings of [16], [21]. Sharma et al. [11], documented significant reduction in Neutrophilic count when rats were treated for 35days with tartrazine at a dose of 0.2g/kg and 0.4g/kg bodyweight. But Himri et al., [16], recorded neutrophilia when rats were treated orally with tartrazine at a dose slightly above ADI (10mg/kg) for 90 days. In addition, Imafidon et al. [21], reported no significant changes in Neutrophilic count when tartrazine was administered in rats at high dose of 10, 20, 40 and 80mg/kg on weekly basis for 4 weeks. Furthermore, the significantly lower values seen in Eosinophils and Basophils observed in this work was contrary to the findings of [11]. Sharma et al.[11], observed no significant change when 0.2 and 0.4g/kg of tartrazine dyes was administered in rats for 35 days. The significantly lower values seen WBCs, Neutrophils,

Eosinophils and Basophils could be as a result of cytotoxic and immunosuppressive effect of tartrazine induced by oxidative molecules such as aryl amines, superoxides and N-hydroxyl groups derived from metabolism of these azo dyes in the liver or intestine by bacteria. These molecules could have also weakened the cell membrane defense mechanism, thereby paving way for oxidative stress associated derangements including cell death.

In addition, the significantly higher value seen in Lymphocyte in the acute study collaborates with the reports of [11], [30]. Sharma et al.[11], documented that tartrazine at a dose of 0.2g/kg and 0.4g/kg bodyweight induced lymphocytosis when rats were treated for 35days. Contrary to our findings, El-Golli et al.[20], observed significant reduction in Lymphocytes when tartrazine was administered in male adult rats at a high dose of 300mg/kg bodyweight for 30 days. In addition, Imafidon *et al.*, [21], recorded no significant changes in Lymphocytes when tartrazine was administered in rats at high doses of 10, 20, 40 and 80mg/kg on weekly basis for 4 weeks. When Monocyte was considered, the significant increase in Monocyte contradicts the findings of [11]. Sharma et al.[11], observed no significant change when 0.2 and 0.4g/kg of tartrazine dyes was administered in rats for 35 days. The increase in Lymphocyte and Monocyte could be as a result of physiological inflammatory or immunologic response by the body system to the presence of xenobiotics (dye) or due to cellular derangements induced by the dye.

In the chronic study, when Neutrophil and Lymphocyte were considered, significantly higher and lower levels were observed in Neutrophils and Lymphocytes respectively in chronically treated rats for 30, 60 and 90 days. Our findings contradict the findings Himriet al.[16], who reported no significant change in Neutrophils and Lymphocytes when tartrazine was fed orally to albino rats at ADI doses (7.5mg/kg) for 90 days. More so, when Eosinophils and Basophils were considered in the chronic study, significantly lower levels were seen in Eosinophils and Basophils of chronically treated rats for 30 days (and Basophils for 90 days treated rats). Our present findings after 30 days of chronic treatment contradict the records of [31]. However, the significantly higher levels seen in Eosino of chronically treated rats for 60 days is in line with the findings of[31]. Moutinho et al. [31], observed significant increase in eosinophil at ADI doses when tartrazine was administered in rats.

In addition, when Monocytes was considered, significantly lower levels were seen in chronically treated rats after 30, 60 and 90 days. This finding contradicts the reports of [16]. Himri et al. [16], recorded no significant difference in monocyte when tartrazine was administered orally at ADI doses in albino rats for 90 days. Furthermore, WBCs indicated significant increase in chronically treated rats after 60 and 90 days. However, our present finding contradicts the records of [15], [16], [32]. Hashem et al. [15], stated that dose of tartrazine as high as 315mg/kg administered in albino rats for 4 weeks did not induce any change in WBCs. Himri et al. [16], also reported no significant difference in WBCs when tartrazine was administered orally at ADI doses (7.5mg/kg) in albino rats for 90 days but Halel [32], recorded significant decrease in WBCs when rats were fed with 0.5mg/kg of tartrazine supplemented with 10mg of sodium nitrite (NaNO_3).

The significantly lower levels seen in Neutrophils, Monocytes, Eosinophils and Basophils of treated rats could be as a result of cell deaths due to disrupted cellular membrane induced by aryl amines, superoxides and other reactive oxygen species, produced through azo bond degradation in the liver or intestine. However, the significantly higher levels of Lymphocytes and WBCs observed in the chronic study could be due to physiological inflammatory or immunological response in an attempt in limiting the induced cellular damages. More so, the significantly higher levels of Lymphocytes fraction observed could also have accounted for the significant increase in WBCs seen in the treated rats compared to the control rats.

Furthermore, when HCT, HB, RBCs and PLTs were considered, significant differences were not seen after 30, 60 and 90 days of chronic treatment. The results of RBCs, HCT and HB obtained in the chronic study support the reports of [12], [16] but contradicts the records of [32]. Mehedi et al. [12], reported that low doses (0.1%) of tartrazine administered daily for 13 weeks did not induce any significant change in HCT, HB and RBCs in albino rats. Also, Himri et al. [16], also reported no significant difference in HCT, HB and RBCs when tartrazine was administered orally at doses of 7.mg/kg and 10mg/kg in albino rats for 90 days. However, Halel [32], recorded significant decrease in RBCs, HB and HCT when rats were fed with 0.5mg/kg of tartrazine supplemented with 10mg of sodium nitrite (NaNO₃). The non-significant differences seen in RBCs, HB and HCT could be attributed to maintenance of the RBCs membrane integrity thereby preventing changes in the RBCs shape. Changes in the cell shape of RBCs have been shown to enhance the destruction and removal of RBCs from the peripheral circulation by the reticulo-endothelial system.

Finally, the non-significant differences seen in PLTs in the chronically treated rats after 30, 60 and 90 days at ADI doses, contradicts the report of [16]. Himri *et al.*, [16], observed significantly lower levels of PLTs when tartrazine was administered orally at ADI doses (7.5mg/kg) in albino rats for 90 days. They further reported no significant change in platelet when the dose was increased to 10mg/kg bodyweight. The non-significant difference observed in PLTs could be attributed to the capacity of the anti-oxidant system to mop up completely reactive oxygen species (ROS) associated with micro-tears on cells and cell dead. More so, changes in the cell shape of PLTs have been shown to enhance the destruction and removal of PLTs from the peripheral circulation by the reticulo-endothelial system.

5. CONCLUSION

In acute toxicity study, the administration high doses of tartrazine induced decreased RBCs, HB, HCT, WBCs, Eosinophils and Neutrophils as well as an increased PLTs, Lymphocytes and Monocytes. However, in the chronic treatment, WBCs were increased after 60 and 90 days of chronic treatment at ADI doses while increase in Lymphocytes were seen after 30, 60 and 90 days. Neu and Mono indicated significant lower levels after 30, 60 and 90 days of chronic treatment while indicated significant decrease after 30 and 60 days. HCT, HB and PLTs showed no significant difference after 30, 60 and 90 days of chronic treatment at ADI doses. The results obtained indicate that high doses of tartrazine above the recommended ADI induced severe haematological derangements. However, the chronic study did not affect HCT, HB and PLTs but mild alterations were in WBCs, Lymphocytes, Neutrophils, Eosinophils and Basophils after 60 and 90 days of treatment.

6. RECOMMENDATION

It is advised that high doses of tartrazine in foods or food products should be avoided completely. More so, because of the mild alterations seen in the chronic study, it is also advised that duration far above 90 days should be considered in further studies.

7. LIMITATION OF THE STUDY

The duration of the chronic aspect of this study was not more than 90 days. Moreover, our present findings were in rats and therefore cannot be directly interpreted that these effects observed in rats will be exactly and/or physiologically the same in humans. Therefore, our findings are subject to further research and verification especially in humans.

CONSENT

Not applicable

ETHICAL APPROVAL

We hereby declare that the Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Rivers State University research/ethics committee with file No: RSU/CV/APU/74/VOL.VIII/104.

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ABBREVIATIONS

ADI	=	ACCEPTABLE DAILY INTAKE
FBC	=	FULL BLOOD COUNT
Hb	=	HAEMOGLOBIN
HCT	=	HAEMATOCRIT
PLT	=	PLATELET
RBCs	=	RED BLOOD CELLS
WBCs	=	WHITE BLOOD CELLS